9-1-1: HSCs Respond to Emergency Calls

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In this issue of *Cell Stem Cell*, Zhao et al. (2014) and Schürch et al. (2014) describe two new stem cell mechanisms underlying protective responses to infection. In response to inflammatory signals, HSPCs and MSCs produce cytokines that stimulate HSC mobilization and differentiation toward innate immune cells at the expense of adaptive immune lineages.

Adaptive immunity is mediated by lymphocytes, which are relatively long lived cells and can replenish themselves by division. In contrast, bone marrow hematopoietic stem cells (HSCs) must constantly produce appropriate numbers of nonlymphoid innate effector cells for host defense. Moreover, they must be able to quickly ramp up the process in response to infections. While this phenomenon has long been known and is assumed to promote organismal survival (Metcalf, 1971), two studies appearing in this issue of *Cell Stem Cell* provide new insights regarding the underlying mechanisms (Zhao et al., 2014; Schürch et al., 2014).

Some pathogens perturb blood cell formation by actually infecting cells within the bone marrow; however, the focus of the recent studies is on processes commonly referred to as "emergency" or "demand-adapted" hematopoiesis (Takizawa et al., 2012), which can be modeled by bacterial or viral infection in mice. Infection simulated with injections of *E.-coli*-derived lipopolysaccharide (LPS), a membrane polysaccharide expressed in all Gram-negative bacteria, would seem to be a simple model of emergency hematopoiesis, but numerous host responses come into play and can be difficult to dissect. LPS is a ligand for Toll-like receptor 4 (TLR4), which is expressed on immune cells and stimulates their activation. Hematopoietic stem and progenitor cells (HSPCs) are also known to express functional TLRs. Indeed, within 1 hr after injection, LPS permeates the bone marrow and binds to TLR4 on HSPCs (Nagai et al., 2006). TLR4 activation stimulates hematopoietic stem cell cycle entry, mobilization to organs such as the spleen, evacuation of B lineage lymphoid cells from the marrow, and redirection of progenitors to nonlymphoid fates. The latter phenomenon can be demonstrated with purified cells maintained under defined culture conditions (Nagai et al., 2006), and the overall outcome is myeloid cell production at the expense of lymphopoiesis.

Engaging TLR on individual progenitors might be sufficient to initiate their division and redirection toward generation of innate effector cells. However, in other circumstances, bystanders are probably recruited via locally released cytokines. Paracrine responses to LPS have been documented in vivo and nonhematopoietic stromal elements can also be sources of factors (Takizawa et al., 2012).

Baltimore and colleagues have now used an NF-κB reporter system to confirm that functional TLR4 as well as TLR2 are present on hematopoietic stem cells (HSCs) and HSPCs (Zhao et al., 2014). Of even greater importance, a single-cell proteomics approach revealed that subsets of short-term HSCs and multipotent progenitors make large amounts of cytokines in response to stimulation with TLR ligands. Indeed, they were more efficient in producing growth and differentiation factors than mature cells were. Furthermore, mouse models with both reduced and exacerbated NF-kB signaling were used to show that the amounts of cytokines produced could be dialed up and down with changes in NF-κB pathway activity. Neutralization and knockout experiments indicated that GM-CSF, TNF, and especially interleukin-6 (IL-6) accounted for most of the escalated nonlymphoid cell production.

IL-6 is a multifunctional cytokine, and neutralization of its receptor represents effective therapy for many inflammatory diseases. Coming from a completely different direction, Offenbein and colleagues found another way that IL-6 participates in emergency hematopoiesis (Schürch et al., 2014) (Figure 1). Again, the study began with a model system. Cytotoxic T lymphocytes (CTL) from T cell receptor transgenic mice were isolated and activated by transfer to other transgenic mice with ubiquitous expression of the complementary antigen. There was a modest but consistent myelopoietic response in the recipient mice that the investigators used to their advantage. They assumed correctly that the CTL produced interferon gamma (IFNγ), which in turn drove myelopoiesis. However, when chimeric mice generated from transplanting antigen-specific CTLs into a reactive host were used, they discovered an unexpected mechanism. That is, radioresistant, nonhematopoietic cells with functional IFNγ receptors, rather than myeloid progenitors, were responding to the CTL-produced factor. A bone marrow fraction that was clearly induced by IFNγ contained mesenchymal stem cells (MSCs), and they appeared to predominantly produce IL-6. Indeed, IFNγ did not cause emergency myelopoiesis in IL-6-deficient mice.

While these findings were also replicated in viral infection models, some aspects should be clarified in future studies. For example, IL-6 can block the earliest steps in lymphopoiesis (Maeda et al., 2005) but there were no signs that this lineage was affected in CTL-injected animals. Also, levels of two transcription factors known to drive myelopoiesis, C/EBPα and Runx1, actually declined in hematopoietic cells of IFNγ-treated mice.

Interferons have been extensively studied with respect to hematopoieisis, but typically in the context of direct effects on HSPCs. Documented responses include the activation of primitive cells that could have survival value, but persistent stimulation during chronic infections is generally harmful (Baldridge et al., 2011). Given the extensive clinical use of IFNs, it will be important to identify situations where IL-6 production by MSCs could be important. It is expected that different pathogens can alter blood cell formation in many other ways. For example, in *E. muris* infection, it is $CD4^+$ T cells within bone marrow that utilize TLR/MyD88 pathway stimulation to produce IFNγ that in turn promotes hematopoetic progenitor cell expansion (Zhang et al., 2013).

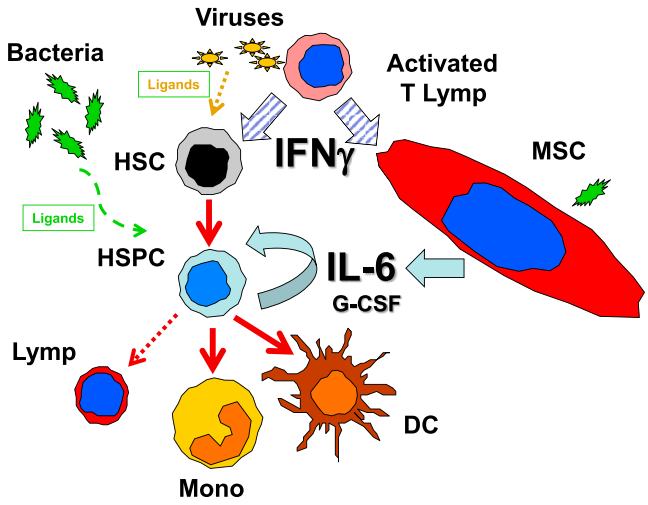

Figure 1. Bone Marrow Response Pathways to Pathogens Converge on Interleukin-6

Hematopoietic stem and progenitor cells (HSPCs) were known to be directly and indirectly influenced by infections. We now know that they can make and respond to their own growth and differentiation factors such as IL-6. As another mechanism, activated T cells can produce interferon that causes mesenchymal stem cells (MSCs) to make IL-6. These and other processes promote production of innate effector cells such as monocytes (Mono) and dendritic cells (DC) while lymphopoiesis is inhibited. Meanwhile, hematopoietic stem cells (HSCs) can be driven into cycle and mobilized to other tissues.

The studies by Zhao et al. and Schürch et al. demonstrate how technical advances and innovation can be used to dissect complicated processes.

Although extremely rare, stem and progenitor cells can be divided into subsets according to phenotypes and functions (Copley et al., 2012). Until recently, this heterogeneity could be fully appreciated only with tedious single-cell transplants. Now, barcoding and proteomics approaches can provide new insight into the nature and significance of that diversity. It is remarkable that individual HSPCs in the Baltimore study differed with respect to numbers, amounts, and nature of cytokines made. Perhaps the progenitors are poised to respond with a repertoire of outcomes, thus providing protection from a large assortment of pathogens.

Immunostaining of marrow sections is difficult, but a number of labs can now localize cytokine-producing cells in relation to components of HSPC niches (Morrison and Scadden, 2014). Thus, an immediate question is where IL-6-producing cells reside in marrow of normal and infected animals. The authors of these new studies suggest they will be in defined niches where local cytokine concentrations can become high. One has to be impressed at the growing number of ways the marrow can respond to life-threatening infections.

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